



ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZR Fecal DNA MidiPrep™

Catalog No. **D6110**

Highlights

- Rapid method for the isolation of inhibitor-free, PCR-quality DNA from fecal samples in as little as 25 minutes including humans, birds, rats, mice, cattle, etc.
- State-of-the-art, ultra-high density **BashingBeads™** are fracture resistant and chemically inert.
- Can be used with any bead mill, disrupter, or vortex that can accommodate standard 50 ml conical centrifuge tubes.
- Omits the use of organic denaturants as well as proteinases.

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Satisfaction of all Zymo Research products is guaranteed. If you should be dissatisfied with this product please call 1-888-882-9682.

Product Contents

ZR Fecal DNA MidiPrep™ (Kit Size)	D6110 (25 preps.)	Storage Temperature
ZR BashingBead™ Lysis/Filtration Tubes	25	Room Temp.
Fecal DNA Binding Buffer	2 x 100 ml	Room Temp.
DNA Pre-Wash Buffer*	15 ml	Room Temp.
Fecal DNA Wash Buffer	50 ml	Room Temp.
DNA Elution Buffer	16 ml	Room Temp.
Zymo-Spin™ V-E Columns w/ Zymo-Midi Filters™	25	Room Temp.
Zymo-Spin™ IV-HRC Spin Filters (Green Tops)	50	Room Temp.
Collection Tubes	100	Room Temp.
Instruction Manual	1	Room Temp.

Note - Integrity of kit components is guaranteed for up to one year from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide maximal performance and reliability.

* A precipitate may have formed in the DNA Pre-Wash Buffer during shipping. To completely resuspend the buffer, incubate the bottle at 30 – 37 °C for 30 minutes and mix by inversion. DO NOT MICROWAVE.

Specifications

- **Format** – Bead Beating, Spin/Vacuum Filtration, and Spin Column Purification
- **Sample Sources** – Bacterial, protist, as well as host DNA can be effectively isolated from a ≤375 mg sample of mammalian feces.
- **DNA Purity** – High quality, humic/fulvic-free DNA is eluted with **DNA Elution Buffer** making it perfect for PCR. $A_{260}/A_{280} \geq 1.8$, $A_{260}/A_{230} \geq 2.0$
- **DNA Size Limits** – Capable of recovering genomic DNA sized fragments from 100 bp to ≥ 40 kb. Typical fragment sizes range from 25 kb-35 kb. If present, parasitic and viral DNA will also be recovered.
- **DNA Recovery** – Typically, up to ~125 µg total DNA is eluted into ≥150 µl **DNA Elution Buffer** per sample.
- **Equipment** – Centrifuge, Vacuum Source and Manifold, Microcentrifuge, Cell Disrupter/Pulverizer w/ 50 ml Tube Adapter

For purification of up to 25 µg DNA/prep use the **ZR Fecal DNA Kit™ (D6010)**. For high throughput purification (96-well) use the **ZR-96 Fecal DNA Kit™ (D6011)**.

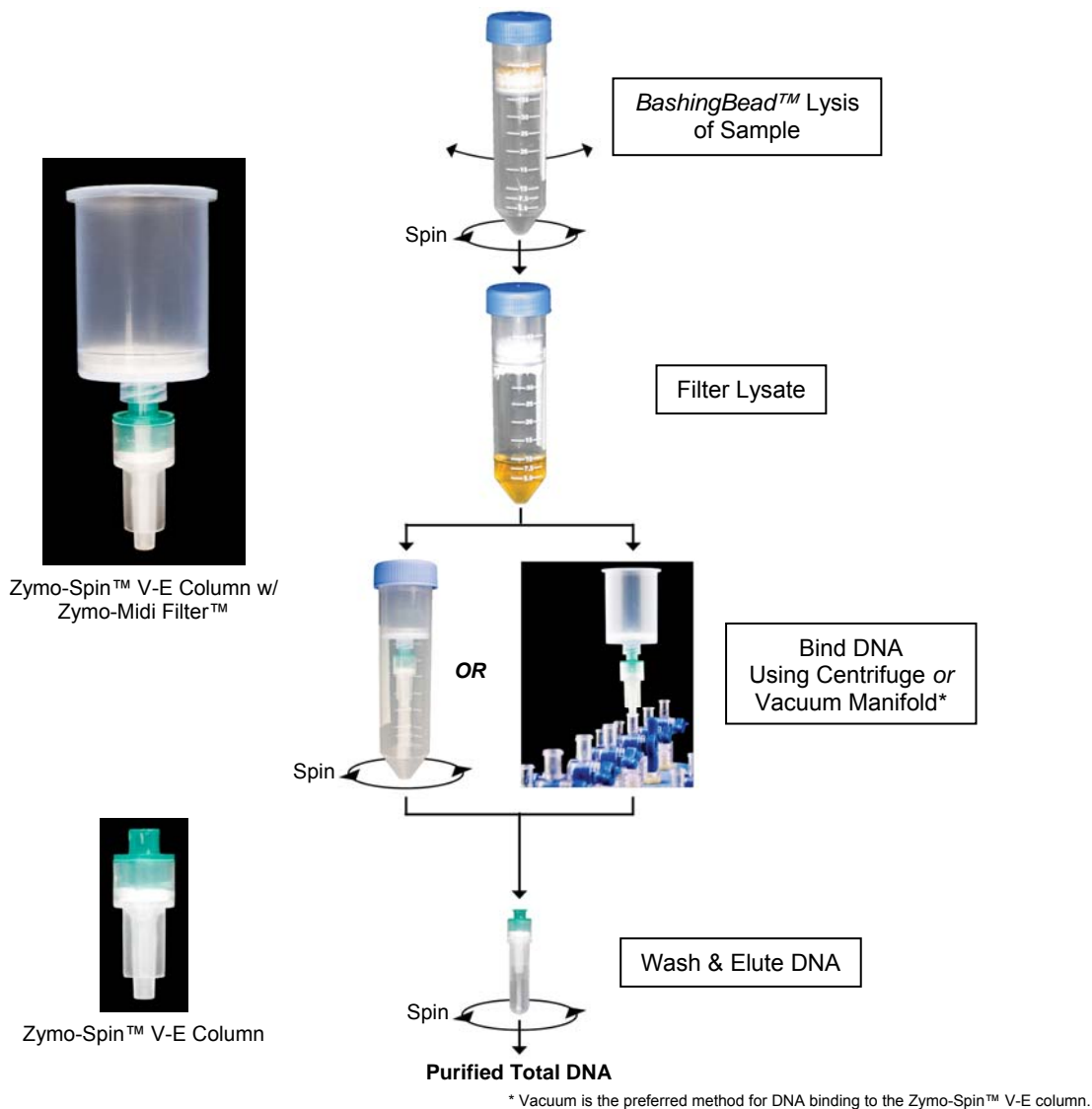
Note - ™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

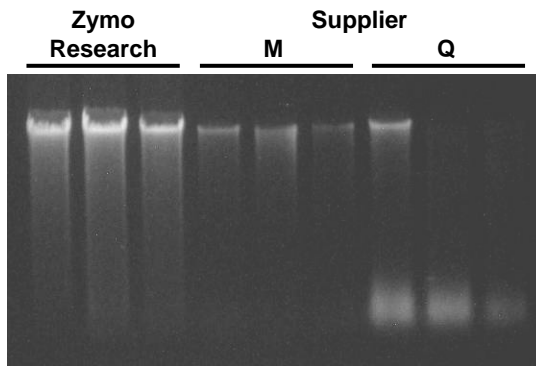
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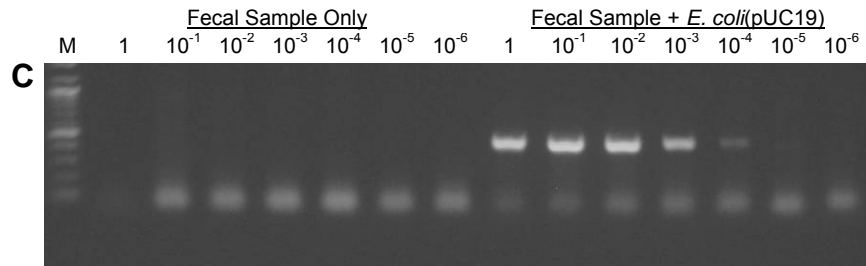
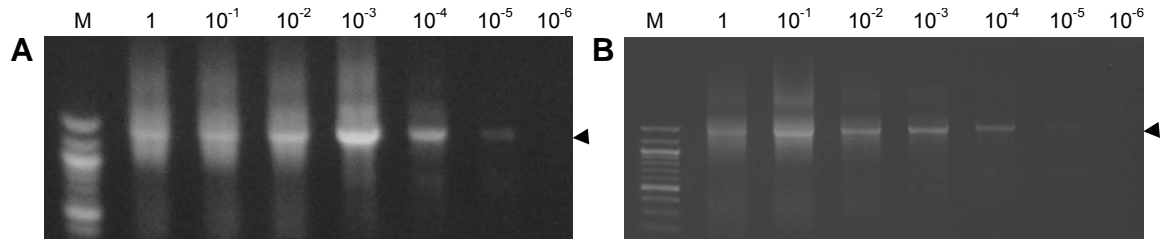
Product Description

The **ZR Fecal DNA MidiPrep™** is designed for the simple, rapid isolation of inhibitor-free, PCR-quality DNA from a variety of sample sources including humans, birds, rats, mice, cattle, etc. The procedure is easy and can be completed in as little as 25 minutes: fecal samples (≤ 375 mg each) are added directly to a **ZR BashingBead™ Lysis/Filtration Tube** and rapidly and efficiently lysed by bead beating without the use of organic denaturants or proteinases. The DNA is then isolated and purified using our *Fast-Spin* column technology and is ideal for downstream molecular-based applications including PCR, arrays, genotyping, etc. The DNA is also well suited for methylation profiling purposes using any of our **EZ DNA Methylation Kits**. A schematic of the **ZR Fecal DNA MidiPrep™** procedure is shown below.





Comparison of DNA yields from rat feces using the **ZR Fecal DNA Kit™** and kits from suppliers M and Q. Equivalent amounts of feces were processed using each kit and then equal volumes of eluted DNA were analyzed in a 0.8% (w/v) agarose/ethidium bromide gel. Samples were processed in triplicate.



PCR of DNAs from rat and human fecal samples isolated with the **ZR Fecal DNA Kit™**. **Panels A and B** show the results of PCR with DNA isolated from rat and human fecal samples, respectively, using primers specific for prokaryotic 16S rRNA. **Panel C** shows the results of PCR of DNA isolated from human feces with and without the addition of *E. coli* containing pUC19 plasmid DNA (indicated at the top of the image) using primers specific for the pUC19 sequence. In each case, amplicons were analyzed in a 1.5% (w/v) agarose / ethidium bromide gel using a UV imager. Numbers above each lane of the gel images are the volumetric equivalent (in μ l) of eluted DNA (100 μ l) used for PCR. Arrows mark the relative migration of amplicons in the gels, and M is a 100 bp DNA ladder (NEB).

For **Technical Assistance**, please contact those at **Zymo Research's Technical Department** at 1-888-882-9682 or E-mail to tech@zymoresearch.com.

Protocol

Before Starting: **Zymo-Spin™ IV-HRC Spin Filters** (green tops) need to be prepared prior to use by: 1) snapping off the base, 2), inserting into a **Collection Tube**, and 3), spinning in a microcentrifuge at exactly 8,000 x g for 3 minutes.

1. Add up to 375 mg of fecal sample to the bead/filter chamber of a **ZR BashingBead™ Lysis/Filtration Tube**. Add 6 ml **Fecal DNA Binding Buffer** to the sample, cap tube¹, and process.

*(To prevent the **Lysis Solution** from leaking into the bottom of the 50 ml tube, place the **ZR BashingBead™ Lysis/Filtration Tube** on its side prior to processing).*

2. Secure in a bead beater fitted with a 50 ml tube holder assembly (see page 6) to process samples. Optimization of processing time/speed will be necessary for complete sample lysis.

Processing times may be as little as 40 seconds when using high-speed cell disrupters (e.g., FastPrep®-24, Geno/Grinder®, page 6). See manufacturer's literature for operating information.

3. Centrifuge the **ZR BashingBead™ Lysis/Filtration Tube** in a centrifuge at $\geq 3,000 \times g$ ($5,000 \times g$ max.) for 5 minutes.
4. Filter the entire mixture from Step 3 using a **Zymo-Spin™ V-E Column/Zymo-Midi Filter™** assembly mounted on a vacuum manifold^{2,3} (see diagram on page 2) with a vacuum source set at ≥ 600 mm Hg.
5. Disconnect the **Zymo-Spin™ V-E Column/Zymo-Midi Filter™** assembly and transfer the **Zymo-Spin™ V-E Column** to a **Collection Tube**. Spin the column at $10,000 \times g$ for 1 minute in a microcentrifuge⁴, then add 300 μ l **DNA Pre-Wash Buffer** to the column and spin at $10,000 \times g$ for 1 minute. Discard the flow through.
6. Add 400 μ l **Fecal DNA Wash Buffer** to the column and centrifuge at $10,000 \times g$ for 1 minute. Discard flow through and repeat wash step.
7. Transfer the **Zymo-Spin™ V-E Column** to a 1.5 ml microcentrifuge tube and add 150 μ l **DNA Elution Buffer** directly to the column matrix⁵ and allow column to stand for 1 minute at room temperature. Centrifuge at $10,000 \times g$ for 1 minute to elute the DNA⁶.
8. Transfer the eluted DNA from Step 7 to a prepared **Zymo-Spin™ IV-HRC Spin Filter** (green top) (see above) in a clean 1.5 ml microcentrifuge tube and centrifuge at exactly $8,000 \times g$ for 1 minute.

The filtered DNA is suitable for PCR, other downstream applications, or storage.

Notes:

¹ Cap tube tightly to prevent leakage.

² Be careful to avoid the pelleted material at the bottom of the tube when transferring the supernatant.

³ Alternatively, the **Zymo-Spin™ V-E Column/Zymo-Midi Filter™** assembly can be placed in a 50 ml tube and centrifuged at $2,000 \times g$ max. for 5 minutes. Filtration of the entire mixture will require several spins. Empty the flow through from the tube after each spin. **CAUTION: Make sure the connection between the column and filter is secure (finger tight) prior to centrifugation!**

⁴ Leave the rotor cover off the microcentrifuge if clearance with the column tops is a problem.

⁵ DNA yields can be increased by performing a second elution and pooling the eluates.

⁶ In some cases a brown-colored pellet may form at the bottom of the tube after centrifugation. Avoid this pellet when collecting the eluted DNA.

Ordering Information

Product Description	Catalog No.	Kit Size
ZR Fecal DNA Kit™	D6010	50 preps.
ZR-96 Fecal DNA Kit™	D6011	2x96 preps.
ZR Fecal DNA MidiPrep™	D6110	25 preps.

For Individual Sale	Catalog No.	Amount
ZR BashingBead™ Lysis/Filtration Tubes (50 ml) w/ 0.5 mm Beads	S6010	25 tubes
Fecal DNA Binding Buffer	D6010-1-100	100 ml
DNA Pre-Wash Buffer	D3004-5-15	15 ml
Fecal DNA Wash Buffer	D6010-2-50	50 ml
DNA Elution Buffer	D3004-4-16	16 ml
Zymo-Spin™ V-E Columns w/ Zymo-Midi Filters™	C1021-25	25 columns/filters
Zymo-Spin™ IV-HRC Spin Filters (Green Tops)	C1010-50	50 filters
Collection Tubes	C1001-50	50 tubes
	C1001-500	500 tubes
	C1001-1000	1,000 tubes

Compatible Lysis Instruments...

Bullet Blender™



NEXT >>> ADVANCE

Homogenize tissue or disrupt/lyse cells in minutes. **The Bullet Blender™**, is a vortexer (at a low setting), a cell disruptor and a tissue homogenizer (at a high setting) all in one unit. No parts contact the samples, eliminating any possibility of cross contamination.

Description	Cat No.
DX50B Bullet Blender™ Blue 50 Accommodates 9 x 50 ml tubes. Features fan cooling.	S6007-1

FastPrep®



The **FastPrep®-24 Instrument** is a unique, high-speed benchtop homogenizer that employs a powerful, proprietary technology for the rapid lysis of almost any sample in 40 seconds or less. The FastPrep® Instrument makes it possible to isolate DNA, RNA and protein from sources that are virtually impossible to lyse without the use of its rapid reciprocating motion.

Description	Cat No.
FastPrep® Instrument	S6005
BigPrep™ Attachment Accommodates 2 x 50 ml tubes.	S6005-4



2000 Geno/Grinder®



The **2000 Geno/Grinder Instrument** is a unique instrument that provides vigorous up-and-down grinding/pulverizing action. The Geno/Grinder instrument makes it possible to prepare plant materials such as seeds, stems, roots, leaves, and certain animal tissue. Can accommodate (2) 96-well plates/blocks for high-throughput sample processing.

Description	Cat No.
2000 Geno/Grinder Instrument	S6006
50 ml Tube Holder/Cryo Block Assembly Accommodates 12 x 50 ml tubes/block.	S6006-3



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Environmental DNA & RNA Purification Products From Zymo Research

Product	Description	Kit Size	Cat No.
<i>Tough-to-Lyse and Environmental Sample DNA Isolation</i>			
ZR Soil Microbe DNA Kit™ ZR-96 Soil Microbe DNA Kit™ ZR Soil Microbe DNA MidiPrep™	Spin Column Format (up to 25 µg/prep.) 96-Well Format (up to 5 µg/well) Vacuum Filtration, Spin Column Format (up to 125 µg/prep.)	50 preps. 2x96 preps. 25 preps.	D6001 D6002 D6101
ZR Fungal/Bacterial DNA Kit™ ZR-96 Fungal/Bacterial DNA Kit™ ZR Fungal/Bacterial DNA MidiPrep™	Spin Column Format (up to 25 µg/prep.) 96-Well Format (up to 5 µg/well) Vacuum Filtration, Spin Column Format (up to 125 µg/prep.)	50 preps. 2x96 preps. 25 preps.	D6005 D6006 D6105
ZR Fecal DNA Kit™ ZR-96 Fecal DNA Kit™ ZR Fecal DNA MidiPrep™	Spin Column Format (up to 25 µg/prep.) 96-Well Format (up to 5 µg/well) Vacuum Filtration, Spin Column Format (up to 125 µg/prep.)	50 preps. 2x96 preps. 25 preps.	D6010 D6011 D6110
ZR Tissue & Insect DNA Kit-5™ ZR Tissue & Insect DNA Kit-25™ ZR-96 Tissue & Insect DNA Kit™ ZR Tissue & Insect DNA MidiPrep™	Spin Column Format (up to 5 µg/prep.) Spin Column Format (up to 25 µg/prep.) 96-Well Format (up to 5 µg/well) Vacuum Filtration, Spin Column Format (up to 125 µg/prep.)	50 preps. 50 preps. 2x96 preps. 25 preps.	D6015 D6016 D6017 D6115
ZR Plant/Seed DNA Kit™ ZR-96 Plant/Seed DNA Kit™ ZR Plant/Seed DNA MidiPrep™	Spin Column Format (up to 25 µg/prep.) 96-Well Format (up to 5 µg/well) Vacuum Filtration, Spin Column Format (up to 125 µg/prep.)	50 preps. 2x96 preps. 25 preps.	D6020 D6021 D6120
<i>Tough-to-Lyse and Environmental Sample RNA Isolation</i>			
ZR Fungal/Bacterial RNA MicroPrep™ ZR Fungal/Bacterial RNA MiniPrep™	Spin Column Format (up to 5 µg/prep.) Spin Column Format (up to 25 µg/prep.)	50 preps. 50 preps.	R2010 R2014
ZR Plant RNA MiniPrep™	Spin Column Format (up to 25 µg/prep.)	50 preps.	R2024
ZR Tissue & Insect RNA MicroPrep™	Spin Column Format (up to 5 µg/prep.)	50 preps.	R2030